Plasma interleukin-6 is independently associated with mortality in both hemodialysis and pre-dialysis patients with chronic kidney disease

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Chronic inflammation associated with chronic kidney disease predicts all-cause and cardiovascular mortality in hemodialysis patients. Here we sought to evaluate the association between plasma levels of the inflammatory mediator interleukin-6 (IL-6) and mortality and aortic calcification/stiffness in 125 patients at different stages (2-5D) of chronic kidney disease. Using multivariate linear regression, we found that plasma IL-6 was independently associated with C-reactive protein, albumin and the stage of chronic kidney disease, but not the aortic calcification score or pulse wave velocity. During follow-up studies (median of 829 days), 38 patients died, 22 from cardiovascular events. Plasma IL-6 significantly predicted overall and cardiovascular mortality; this association persisted after multiple adjustments or restricting the analysis to pre-dialysis patients. Moreover, IL-6 was a significantly better predictor of mortality than C-reactive protein, albumin or tumor necrosis factor-α. Hence, plasma IL-6 independently predicted overall and cardiovascular mortality in patients at different stages of chronic kidney disease; however, whether lowering plasma IL-6 will affect the outcome of chronic kidney disease will require more direct evaluation.

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KEYWORDS: cardiovascular disease; chronic kidney disease; inflammation; mortality; vascular calcification

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Cardiovascular disease is extremely prevalent in chronic kidney disease (CKD) settings and accounts for the majority of deaths in this population.[1] It has been repeatedly shown that advanced CKD is associated with a state of chronic inflammation, as evidenced by either elevated levels of various pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, etc.) or altered levels of acute-phase proteins (C-reactive protein (CRP), albumin, fetuin-A, etc.).[2–4] In addition to individual genetic predispositions,[5] the uremic state probably increases the peripheral cell release of pro-inflammatory cytokines and slows removal of the latter—resulting in a net increase. Importantly, this chronic inflammation state has been shown to predict all-cause and cardiovascular mortality in hemodialysis patients,[6–9] which suggests that high inflammatory responsiveness to environmental stimuli may determine the mortality risk in this population. However, few studies have evaluated the inflammatory profile of individuals at the earlier CKD stages[10–12] and none of them examined the association of this profile with outcomes in these patients.

Interleukin-6 (IL-6) is a 26 kDa protein produced by the liver. It is considered to be crucial in the acute-phase inflammatory response and promotes lymphocyte activation and proliferation, B-cell differentiation, leukocyte recruitment and regulation of the synthesis of acute phase proteins, fibrinogen, and albumin.[13] Plasma levels of IL-6 are known to be significantly higher in severely ill patients with acute renal failure who die than in those who survive to hospital discharge (independently of sepsis status).[14] In addition, in the elderly [15,16] and CKD patients on dialysis,[17,18] plasma IL-6 levels have been shown to better predict death than IL-1β, TNF-α, CRP or albumin levels do. Hence, the aim of this study in a cohort of patients at different CKD stages (2 to 5D) was to verify the association between plasma IL-6 levels on one hand and mortality and two important cardiovascular surrogate markers (namely aortic calcification and stiffness) on the other.

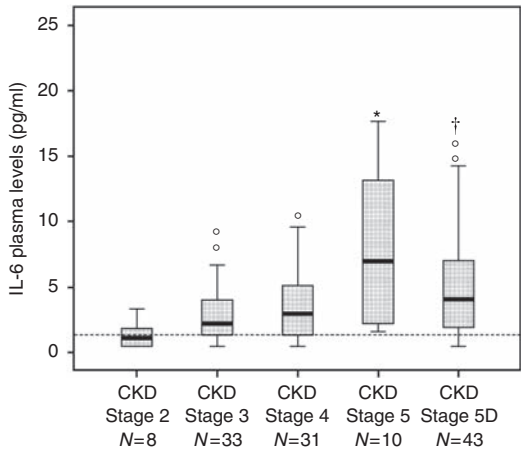


Figure 1 | Plasma IL-6 levels as a function of CKD stage. *$P<0.05$ vs CKD stage 2; †$P<0.05$ vs CKD stages 2 and 3. The dotted line indicates the mean normal value (1.32 pg/ml). CKD, chronic kidney disease.

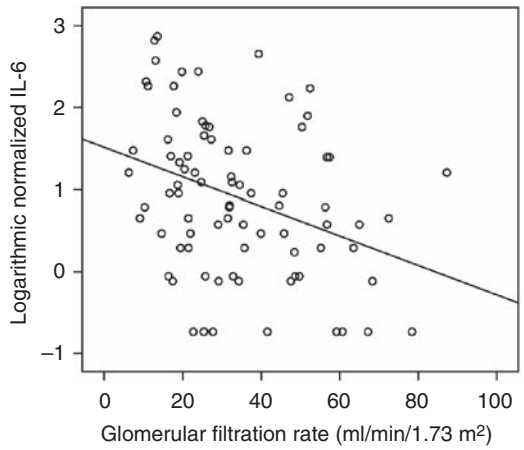


Figure 2 | Linear regression curve. Relationship between log-normalized plasma IL-6 levels and the estimated glomerular filtration rate, for pre-dialysis stage 2–5 CKD patients ($n=82$); $r^2=0.122$; $P=0.001$.

RESULTS

The distribution of IL-6 levels by CKD stage is depicted in Figure 1. The IL-6 levels tended to rise as CKD progressed with the increase becoming statistically significant at CKD stages 5 and 5D. Further examination confirmed an inverse linear relationship between IL-6 levels and the eGFR when the analysis was restricted to pre-dialysis CKD patients at stages 2–5, as shown in Figure 2.

Tables 1 and 2 show the main characteristics of the study population, as a function of the median IL-6 plasma level.

Table 1 | Main demographic and clinical characteristics, as a function of the median plasma IL-6 levels

	All (n=125)	IL-6 ⩽2.97 pg/ml (n=63)	IL-6 >2.97 pg/ml (n=62)	*P*-value
Age (years)	67 ± 12	65 ± 13	70 ± 11	0.031
Male gender *n* (%)	77(62)	37 (59)	40 (64)	0.506
Body mass index (kg/m^2)	28 ± 6	27 ± 6	29 ± 6	0.137
SBP (mm Hg)	154 ± 26	154 ± 25	153 ± 28	0.833
DBP (mm Hg)	81 ± 12	83 ± 12	78 ± 12	0.015
Pulse pressure (mm Hg)	73 ± 23	71 ± 23	75 ± 23	0.305
Diabetes *n* (%)	52 (42)	22 (35)	30 (48)	0.127
Smoking habit *n* (%)[a]	48 (40)	26 (41)	22 (38)	0.708
Presence of CVD *n* (%)	40 (32)	18 (29)	22 (35)	0.407
Framingham risk score	8.0 ± 3.6	7.8 ± 3.6	8.3 ± 3.5	0.397
Current statin use *n* (%)	75 (60)	44 (70)	31 (50)	0.024
Current ACE/ARA-2 inhibitor use *n* (%)	74 (59)	44 (70)	30 (48)	0.015
CKD stage n *(%)*				0.002
2	8 (6.4)	7 (11.1)	1 (1.6)	
3	33 (26.4)	23 (36.5)	10 (16.1)	
4	31 (24.8)	16 (25.4)	15 (24.2)	
5	10 (8)	3 (4.8)	7 (11.3)	
5D	43 (34.4)	14 (22.2)	29 (46.8)	
Central venous access *n* (%)	9 (7.2)	7 (11.1)	2 (3.2)	0.164

Abbreviations: ACE, angiotensin-converting enzyme; ARA-2, angiotensin II type 1 receptor; CKD, chronic kidney disease; CVD, cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure.
Data are means ± s.d. or number (frequency) for binary variables.
[a]n=121.

Table 2 | Main biochemical and vascular measurements as a function of the plasma IL-6 level (relative to the median).

	All (n=125)	IL-6 ⩽2.97 pg/ml (n=63)	IL-6 >2.97 pg/ml (n=62)	*P*-value
IL-6 (pg/ml)	5.25 ± 7.89 (2.97; 1.5–6.1)	1.54 ± 0.76 (1.6; 0.9–1.9)	9.03 ± 9.87 (6.1; 4.1–9.7)	—
Calcium (mmol/l)	2.30 ± 0.18	2.32 ± 0.15	2.27 ± 0.20	0.171
Phosphate (mmol/l)	1.29 ± 0.45	1.23 ± 0.36	1.34 ± 0.52	0.198
Intact-PTH (pg/ml)	139 ± 142 (81; 46–173)	119 ± 116 (71; 45–142)	161 ± 163 (119; 58–194)	0.101
Hemoglobin (g/l)	12.0 ± 1.7	12.4 ± 1.4	11.5 ± 1.8	0.003
Albumin (g/l)	37 ± 6	39 ± 6	35 ± 5	<0.0001
C-reactive protein (mg/l)	10.4 ± 23.1 (3.5; 1.3–8.9)	3.52 ± 7.02 (1.5; 0.6–4.1)	17.4 ± 30.6 (7.4; 3.4–20.1)	<0.0001
TNF-α (pg/ml)	3.99 ± 1.92 (3.6; 2.2–5.1)	3.80 ± 1.79 (3.3; 2.2–5.1)	4.2 ± 2.04 (4.1; 2.5–5.1)	0.193
Total cholesterol (mmol/l)	4.9 ± 1.1	5.0 ± 1.0	4.8 ± 1.2	0.412
HDL cholesterol (mmol/l)	1.34 ± 0.5	1.40 ± 0.5	1.28 ± 0.5	0.180
Triglycerides (mmol/l)	2.05 ± 1.35	1.78 ± 0.1	2.35 ± 1.6	0.024
Aortic calcification score (%)[a]	3.18 ± 3.11 (2.2; 0.8–4.4)	2.72 ± 2.95 (1.5; 0.6–4.0)	3.63 ± 3.22 (2.6; 0.9–5.3)	0.070
PWV (m/s)	15 ± 4	14 ± 3	16 ± 4	0.007

Abbreviations: HDL, high-density lipoprotein; PTH, parathyroid hormone; PWV, pulse wave velocity; TNF, tumor necrosis factor. Data are means ± s.d. and (median; percentile 25–75) for variables with non-Gaussian distribution.
[a]n=108.

Table 3 | Multivariate linear regression analysis—variables independently associated with plasma IL-6 levels (log-normalized)

	β (95% CI)	*P*-value
CKD stage	0.182 (0.085–0.279)	<0.0001
Albumin	−0.038 (−0.060 to −0.016)	0.001
C-reactive protein (log-normalized)	0.380 (0.288–0.473)	<0.0001

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; PTH, parathyroid hormone.
Variables entered in the model: C-reactive protein, albumin, CKD stage, hemoglobin, current ACE/ARA-2 inhibitor use, triglycerides, intact-PTH (log-normalized), calcium; r^2 for the model=0.56.

Patients with plasma IL-6 >2.97 pg/ml were significantly older, had lower diastolic blood pressure, were less likely to be treated with statins and angiotensin-converting enzyme (ACE)/angiotensin II type 1 receptor (ARA-2) inhibitors and were more likely to suffer from late-stage CKD (that is, 5 and 5D). These patients had higher CRP and triglyceride levels and significantly lower hemoglobin and albumin levels. They had higher pulse wave velocity (PWV) values and tended to have higher aortic calcification scores.

In univariate linear regression analyses, CRP ($r^2=0.435$; $P<0.0001$), triglyceride ($r^2=0.042$; $\beta=0.156$; $P=0.024$) and intact PTH levels ($r^2=0.035$; $P=0.039$), and the CKD stage ($r^2=0.144$; $P<0.0001$) were directly associated with higher plasma IL-6 levels. Conversely, albumin ($r^2=0.198$; $P<0.0001$), hemoglobin ($r^2=0.084$; $P=0.001$) and calcium levels ($r^2=0.034$; $P=0.041$) and ongoing use of ACE/ARA-2 inhibitors ($r^2=0.064$; $P=0.005$) and statins ($r^2=0.028$; $P=0.06$) were inversely associated with plasma IL-6 levels. There was no significant linear correlation between plasma IL-6 levels and the aortic calcification score or the PWV. In a multivariate linear regression analysis, only CRP, albumin and the CKD stage were found to be independently associated with plasma IL-6 levels, as detailed in Table 3.

During the follow-up period (mean: 786 ± 301 days; median: 829; range: 10–1119), 38 patients died (22 from CV events (unknown cause or sudden death ($n=13$), stroke ($n=3$), myocardial infarction ($n=1$), congestive cardiac failure ($n=3$), ventricular arrhythmias ($n=2$)), eight from infectious diseases and eight from other causes). In a crude analysis, the IL-6 plasma level predicted overall (Figure 3a) and cardiovascular mortality (Figure 3b) ($P=0.002$ and $P=0.003$ in the log-rank comparison between the curves, respectively). Table 4 shows the results of the Cox regression analyses, which confirmed that plasma IL-6 was still a predictor of mortality (either entered as a continuous variable or stratified by the median) after adjustment for other variables that have also been shown to significantly influence the mortality risk in this population (age, per 1 year increase, RR = 1.05; $P=0.004$; hemoglobin, per 1 g/l increase, RR = 0.73, $P=0.002$; aortic calcification score, per 1 unit increase, RR = 1.24, $P=0.0003$; CKD stage, RR = 1.73, $P=0.010$). Similar results were found when considering IL-6 levels stratified by quartile (3rd quartile: RR = 4.4, 95% CI = 1.25–15.5, $P=0.021$ and 4th quartile: RR = 6.3, 95% CI = 1.8–21.9, $P=0.004$). An additional Cox regression model including the calculated propensity score (by median and to better adjust for confounders, as detailed in the methodology section) confirmed IL-6 as an independent predictor of overall mortality, both when entered as a continuous variable (RR = 1.588, 95% CI = 1.069–2.36, per 1 s.d. increment) or categorized by the median (RR = 2.23, 95% CI = 1.030–5.064, for IL-6 > 2.97 pg/ml). When restricting the analysis to stage 2–5 pre-dialysis CKD patients, plasma IL-6 levels remained a predictor of overall and cardiovascular mortality, in both crude analysis (log-rank $P=0.009$ and $P=0.02$, respectively) (Figure 4) and the Cox regression analysis (Table 5). An additional analysis confirmed plasma IL-6 as a better predictor of death than the three other acute inflammatory response markers assayed in the study (CRP, albumin and TNF-α), when either considering all patients (Table 6) or restricting the analysis to pre-dialysis CKD patients at stages 2–5 (data not shown).

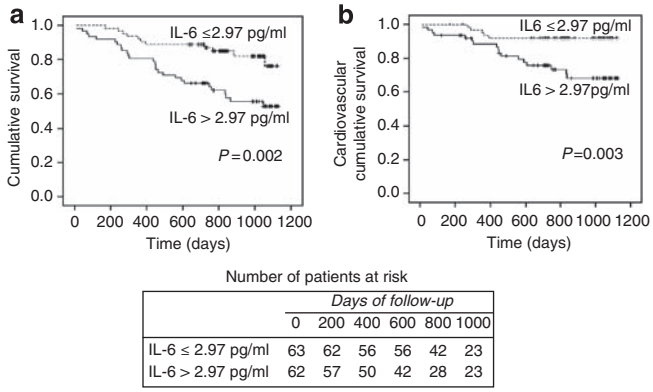


Figure 3 | Crude analyses of mortality. (**a**) Kaplan–Meyer estimates of overall mortality for all patients ($n=125$) as a function of median plasma IL-6 levels. (**b**) Kaplan–Meyer estimates of cardiovascular mortality for all patients ($n=125$) as a function of median plasma IL-6 levels.

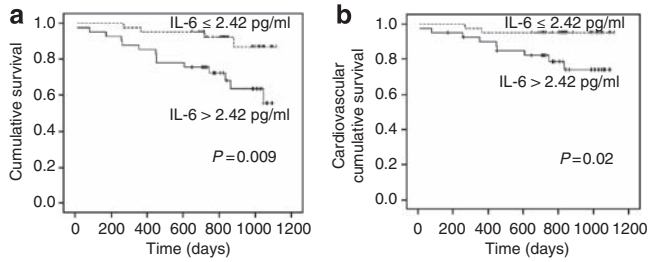
Table 4 | Univariate and multivariate Cox regression analysis of risk factors at baseline for all-cause mortality

Models of patient survival (event *n*=38)	RR	95% CI	*P*-value
Unadjusted			
IL-6[a]	2.837	1.406–5.726	0.004
Log-normalized IL-6[b]	1.748[c]	1.258–2.431	0.001
Model 1[d]			
IL-6[a]	2.352	1.035–5.343	0.041
Log-normalized IL-6[b]	1.670[c]	1.113–2.504	0.013

Abbreviations: CI, confidence interval; RR, risk ratio.
[a]IL-6 entered as categorical variable (IL-6 >2.97 pg/ml vs IL-6 ⩽2.97 pg/ml; median).
[b]IL-6 entered as continuous variable.
[c]Summarizing the risk of a 1-s.d. (1.025, that is, 2.787 pg/ml) increment in log-normalized IL-6.
[d]Model 1: adjusted for age, hemoglobin, aortic calcification score, CKD stage.

DISCUSSION

In this study, we showed that plasma levels of IL-6 augment with CKD stage (particularly at CKD stages 5 and 5D).



Number of patients at risk

	Days of follow-up					
	0	200	400	600	800	1000
IL-6 ≤ 2.42 pg/ml	41	41	39	38	29	13
IL-6 > 2.42 pg/ml	41	38	35	32	19	12

Figure 4 | Crude analysis of mortality in pre-dialysis patients. (**a**) Kaplan–Meyer estimates of overall mortality for patients at CKD stages 2–5 ($n = 82$) as a function of median plasma IL-6 levels. (**b**) Kaplan–Meyer estimates of cardiovascular mortality for patients at CKD stages 2–5 ($n = 82$) as a function of median plasma IL-6 levels.

Table 5 | Univariate and multivariate Cox regression analysis of risk factors at baseline for all-cause mortality in pre-dialysis CKD patients stages 2–5 (*n*=82)

Models of patient survival (event *n*=18)	RR	95% CI	*P*-value
Unadjusted			
IL-6[a]	3.945	1.298–11.995	0.016
Log-normalized IL-6[b]	2.04[c]	1.187–3.525	0.010
Model 1[d]			
IL-6[a]	4.20	1.190–14.829	0.026
Log-normalized IL-6[b]	2.118[c]	1.138–3.942	0.018

Abbreviations: CI, confidence interval; CKD, chronic kidney disease; RR, risk ratio.
[a]IL-6 entered as categorical variable (IL-6 >2.42 pg/ml vs IL-6 ⩽2.42 pg/ml; median).
[b]IL-6 entered as a continuous variable.
[c]Summarizing the risk of a 1-s.d. (1.025, that is, 2.787 pg/ml) increment in log-normalized IL-6.
[d]Model 1: adjusted for age and aortic calcification score.

Table 6 | Relative risk for albumin, CRP and IL-6 to mortality in a Cox proportional hazard (*n*=125)

	Hazard ratio (95% confidence interval)			
	Univariate	*P*-value	Multivariate[a]	*P*-value
Albumin	0.959 (0.912–1.009)	0.104	0.929 (0.874–0.986)	0.016
Log-normalized CRP	1.245 (1.004–1.543)	0.046	1.194 (0.943–1.512)	0.140
Log-normalized TNF	1.159 (0.546–2.459)	0.702	0.798 (0.355–1.791)	0.584
Log-normalized IL-6	1.725 (1.251–2.378)	0.001	1.700 (1.158–2.495)	0.007

Abbreviations: CKD, chronic kidney disease; CRP, C-reactive protein; TNF, tumor necrosis factor.
[a]Adjusted variables included age, CKD stage, and aortic calcification score in each biomarker model.

Importantly, plasma IL-6 levels predicted overall and cardiovascular mortality, even after adjusting for several covariates (that is, age, serum hemoglobin levels, aortic calcification score, and CKD stage) or restricting the analysis to the pre-dialysis CKD patients at stages 2–5. Lastly, plasma IL-6's power to predict mortality was greater than three other important biomarkers of inflammation assayed in the study cohort, namely CRP, TNF-α, and albumin.

The pathological pathway underlying the chronic inflammation state associated with uremia is still poorly understood. Even though various dialysis-related factors (for example, poor dialyzer membrane biocompatibility, dialysate contamination, type of vascular access, etc.) may promote a persistent, low-grade inflammatory response, the present data showing augmented plasma IL-6 levels in earlier CKD stages suggest that the role of loss of kidney function or the kidney disease etiology are at least as important in modulating inflammation or inflammation mediators. It is possible that uremia activates inflammation and/or triggers a reduction in the renal clearance of pro-inflammatory cytokines. Moreover, advanced age and diabetic status (both of which are factors known to contribute to sustained inflammatory activity) were not associated with plasma IL-6 levels in the study population, which substantiates the effect of renal dysfunction. In a previous cross-sectional study including CKD stage 5 and 5D patients, individuals with stable angina and normal renal function and healthy controls, it was reported that serum creatinine was the sole independent determinant of plasma IL-6 levels.[11] Conversely, and in contrast to our own findings, the only other study to have evaluated plasma IL-6 levels in patients at earlier CKD stages (3–5) found that this interleukin was significantly elevated in the CKD patients (compared with healthy controls), but that there was no association with the estimated glomerular filtration rate (GFR).[12] This divergence most probably reflects the effects of the latter study's small sample size ($n = 60$) and/or differences in the IL-6 assay and GFR estimation.[12]

The epidemiological association between plasma IL-6 levels and mortality has repeatedly been reported in different sub settings in both the general population[19,20] and hemodialysis patients.[9,14,18,21] However, to the best of our knowledge, this is the first study to report such an association in a cohort including patients at earlier (pre-dialysis) CKD stages. In this respect, there is much evidence to suggest that pre-dialysis CKD patients are also at a greater risk of cardiovascular disease and the associated morbidity and mortality. Notably, most patients with CKD stages 3–5 will die of cardiovascular complications before developing end-stage renal disease.[22] Recent reports indicate that IL-6 might be involved in cardiovascular pathologies—perhaps even directly by affecting the atherosclerotic burden, as high plasma IL-6 levels have been related to the risk of coronary death and major coronary events in patients with unstable angina.[23,24] Furthermore, IL-6 mRNA has been detected in coronary plaque samples obtained from patients having undergone heart transplantation or atherectomy.[25] Accordingly, in an experimental model of Apo-E-deficient mice, it has been shown that the injection of recombinant IL-6 accelerated atherosclerosis.[26] This hypothesis is corroborated by the fact that higher plasma IL-6 levels predict the risk of future myocardial infarction among apparently healthy

men[20] and have been associated with severe congestive heart failure.[25,27] In hemodialysis patients, a positive association has been reported between plasma IL-6 levels and carotid intima-media thickness but not the presence of carotid plaque.[2] Furthermore, in a small peritoneal dialysis cohort, Stompor *et al.*[28] found a significant, positive association between plasma IL-6 levels and PWV. In this study, we found that patients with higher IL-6 levels presented significantly higher PWV and a trend towards a higher aortic calcification score. Nevertheless, the fact that the observations were not maintained in the subsequent linear regression analyses implies that the relationship between IL-6 and these vascular parameters is rather weak and suggests that the main effect of IL-6 on the risk of mortality in this cohort was independent of vascular calcification and stiffness. As the majority of cardiovascular deaths in our cohort were ascribed as unknown cause or sudden death, this might imply a link between plasma IL-6 and plaque rupture/sudden death.

The finding that plasma IL-6 levels were able to independently predict mortality in both advanced and early-stage CKD raises a key question: are there any active measures or interventions that might reset this cytokine imbalance from the very initial stages of CKD onwards? In this respect, it is important to note that some of the drugs that are commonly administered to CKD patients (that is, statins and ACE/ARA-2 inhibitors) have been shown to have major anti-inflammatory effects and also decrease IL-6 levels in both non-renal patients with coronary artery disease[29,30] or essential hypertension[31] and CKD patients.[32,33] In this study, use of statins and ACE/ARA-2 inhibitors was higher in the group with lower IL-6 levels, in agreement with the above hypothesis. Although other factors (for example, different therapeutic approaches, depending on the degree of renal dysfunction) may also have a role, it should be considered that interventional measures targeting a reduction in IL-6 levels could possibly reduce the unacceptably high cardiovascular mortality observed in the uremic population. It is noteworthy that statin administration lowered CRP levels (compared with placebo) but had no impact on outcomes in a diabetic, hemodialyzed population.[34] Furthermore, statin use in non-diabetic hemodialysis patients, decreased CRP levels but did not improve outcomes;[35] however, one must consider that in this final stage of CKD, the vascular damage might have become irreversible.

Limitations of this study include the relatively small sample size and the fact that we relied on a single blood sample (and thus cannot take into account any potential variations over time in IL-6 levels). Nevertheless, in this respect, Pupim *et al.*[36] monitored a cohort of incident hemodialysis patients over 12 months and found that although these individuals had significantly higher plasma IL-6 levels than healthy controls, there were no significant changes in repeated measurements of plasma IL-6 levels over the study period. All trends toward variation over time were dependent on the initial baseline values. It is possible that plasma IL-6 levels behave similarly in the pre-dialysis setting; hence, additional studies evaluating within-subject variation would be of great value. On the other hand, the strengths of this study include the fact that we evaluated the effect of a potentially modifiable condition (inflammatory state) on a hard outcome parameter (mortality) and on two important surrogate cardiovascular markers in patients at different CKD stages.

In conclusion, plasma levels of IL-6 are significantly elevated in advanced CKD and independently predict overall and cardiovascular mortality in a cohort of patients at different CKD stages with greater prediction power than the three other evaluated acute phase proteins (CRP, TNF-α, and albumin). Further studies evaluating the effect of commonly used drugs with proven anti-inflammatory activities (such as statins and ACE/ARA-2 inhibitors) on plasma IL-6 levels and associated outcomes in a CKD setting, particularly in pre-dialysis patients, are warranted.

PATIENTS AND METHODS

Patient selection

Over an 18-month period (from January 2006 to June 2007), a total of 150 Caucasian CKD patients were recruited from the Nephrology Department's clinic at Amiens University Hospital. All patients gave their informed, written consent. The study was approved by the local Institutional Review Board and performed in accordance with the ethical principles of the Declaration of Helsinki.

Included patients had to be above the age of 40 years, with a confirmed diagnosis of CKD (defined as being on hemodialysis or having two previous, estimated creatinine clearances—calculated according to the Cockcroft and Gault formula[37]—with an interval of 3–6 months and values <90 ml/min per 1.73 m^2). Stage 5D CKD patients had been receiving thrice-weekly hemodialysis for at least 3 months. The presence of chronic inflammatory disease, atrial fibrillation, complete heart block, abdominal aorta aneurysm, an aortic and/or femoral artery prosthesis, primary hyperparathyroidism, kidney transplantation or any acute cardiovascular event in the 3 months before screening were non-inclusion criteria. The 125 patients who met all inclusion criteria and had available IL-6 assay results were analyzed. The 25 patients who were not included in the analyses did not differ from the analyzed ones in terms of age, gender, diabetes, previous cardiovascular disease status, body mass index, or CKD stage distribution.

Study protocol

All patients were hospitalized for the day to perform laboratory blood tests, blood pressure measurements, PWV determinations, and multislice spiral computed tomography scanning. Hemodialysis patients were seen on a dialysis-free day or in the morning before the dialysis session. A patient interview focused on comorbidities and the personal disease history (especially previous vascular events). The patients' medical files were reviewed to record any concomitant medications. For descriptive purposes, patients who reported current or past use of insulin and/or oral hypoglycemic drugs were considered to be diabetics. Previous CVD was defined as a history of any of the following events: myocardial infarction, stroke, heart failure, angina pectoris, or surgical procedures for angina or coronary/peripheral artery disease (including percutaneous transluminal angioplasty). The Framingham risk score[38] was calculated for every patient.

Laboratory tests

Blood samples were collected on the same morning, before other investigations. Selected assays were performed after the samples had been frozen and stored at −80°C. Serum calcium, phosphate, cholesterol, hemoglobin, and creatinine were assayed in an on-site biochemistry laboratory using standard auto-analyzer techniques (Modular IIP system, Roche Diagnostics, Basel, Switzerland). Serum intact PTH was determined in a chemiluminometric immunoassay (Liaison N-tact PTH CLIA, Diasorin, Stillwater, USA). TNF-α plasma levels (2.88 pg/ml, ranging from non-detectable to 5.89 pg/ml) and IL-6 plasma levels were determined by ELISAs (R&D Systems, Wiesbaden, Germany). The limit of detection of the IL-6 ELISA is 0.48 pg/ml and the highest reference standard is 1500 pg/ml. Values below the limit of detection were considered to include the minimum detectable concentration (0.48 pg/ml). Values above the highest standard were not included. All data came from a single determination. Normal values in EDTA-plasma were those stated by the manufacturer (mean of $n = 38$: 1.32 pg/ml; range: ND-4.70 pg/ml). CRP (normal value 2.87 mg/l, ranging from non-detectable to 3.0 mg/l) albumin and cystatin C (CysC) serum levels were determined by laser nephelometry (BNProSpec, Siemens Healthcare, Dade Behring, Marburg, Germany). To describe the true GFR as closely as possible, the estimated GFR combining Scr and CysC measurements (CKD-epi) was calculated for all non-dialyzed patients according to the following, recently published 'CKD-epi' equation:[39] $177.6 \times \mathrm{Scr}^{-0.65} \times \mathrm{CysC}^{-0.57} \times \mathrm{age}^{-0.20} \times$ (0.82 if female patient). Patients were then classified into CKD stages, according to the National Kidney Foundation's K/DOQI guidelines.[22]

Pulse wave velocity evaluation

Carotid-femoral PWV was determined automatically with a dedicated, validated device (Complior Colson, Createch Industrie, Massy, France), as previously detailed elsewhere.[40,41]

Multislice spiral computed tomography

To quantify the presence and extent of aortic calcifications, each patient underwent an multislice spiral computed tomography scan. All examinations were performed with a 64-detector CT scanner (Lightspeed VCT, GE Healthcare, Milwaukee, WI, USA). Detailed technical information on the procedure has been provided elsewhere.[40]

Survival

Death records were made prospectively, by considering all patients included at least 20 months before the study end date (March 1st, 2009). Each medical chart was reviewed and the cause of death was assigned by a physician on the basis of available clinical information. For out-of-hospital deaths, the patient's general practitioner was interviewed to gain pertinent information on the cause. Cardiovascular mortality was defined as any death directly related to a cardiovascular system dysfunction (stroke, myocardial infarction, congestive cardiac failure, sudden death, or death from an unknown cause).

Statistical analyses

Data are expressed as the mean ± s.d., median and range or frequency. For analytical purposes, patients were stratified according to the median plasma IL-6 (2.97 pg/ml). Intergroup comparisons were performed using a χ^2-test for categorical variables and the Student's *t*-test or the Mann–Whitney test for continuous variables. Renal function was considered either as the estimated GFR (in analyses restricted to pre-dialysis patients) or the CKD stage entered as a continuous variable (recoded from 2 to 6, with 6 meaning CKD stage 5D patients). Univariate linear regression analyses were performed to evaluate the association between plasma IL-6 and selected demographic, biochemical and clinical variables. Thereafter, a multiple linear regression analysis of the factors selected in the univariate analysis was carried out to identify those independently associated with plasma IL-6. The Kaplan–Meyer actuarial method was used to estimate overall survival for the IL-6 median. The log-rank test was used to compare survival curves. Univariate and multivariate analyses of mortality were performed by using a Cox proportional hazards model of death as a function of IL-6 levels (either categorized by the median or as a continuous variable). In the multivariate analysis, the predefined models included those variables significantly associated with death in univariate analyses. In view of the limited size of the present cohort, supplementary Cox regression analyses were performed including a propensity score adjustment, which considers each individual's probability of exposure to measured, confounding variables (that is, age, CKD stage, hemoglobin, and albumin), as detailed elsewhere.[42] A *P*-value $\leqslant 0.05$ was considered to be statistically significant. All statistical analyses were performed using SPSS (SPSS, Chicago, IL, USA), version 13.0 for Windows (Microsoft Corp, Redmond, WA, USA).

The authors had full access to the data and take the responsibility for its integrity. All authors have read and agree to the paper as written.

DISCLOSURE

All the authors declared no competing interests.

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